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Research Article

COMPARISON OF THE AUTOMATED BLOOD CULTURE SYSTEM VERSUS CONVENTIONAL METHODS FOR CULTURE OF BODY FLUIDS

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ABSTRACT

Presence of microorganisms in sterile body sites can lead to life-threatening infections. For early and accurate diagnosis of these infections, cultures of the fluids have to be done. These cultures cannot always detect the causative agents due to insufficient number or fastidious growing of the probable microorganisms in the material. In this study, we aimed to compare body fluid cultures processed with conventional culture methods and automated culture system retrospectively. Studies have demonstrated that large-volume culture methods for body fluids other than blood increase recovery compared to traditional plated-medium methods. BacT/Alert is a fully automated blood culture system for detecting bacteremia and fungemia. The BacT/Alert system was evaluated for its utility to recover clinically significant bacteria from several body fluids, other than blood. Of the 148 specimens processed, 37 (25.00%) specimen recorded growth of microorganisms by the BacT/Alert bottles while only 7 (4.73%) specimen recorded growth of microorganisms on the plate cultures. The mean time to detection of growth was <24 hours for bacterial growth and 24 to 48 hours for Candida Species. Though there are chances of growth of contaminant microorganisms because of improper collection technique, improper transportation and sample storage. In our study 26 (17.56%) cultures were recorded growth of contaminant with Automated System.

In conclusion, our study shows that inoculation of the sterile body fluid specimens into blood culture bottles and incubation of them in automated blood culture system increase the detection rate of probable causative agents

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INTRODUCTION

Biological body fluids, such as Ascitic fluid, cerebrospinal fluid (CSF), peritoneal fluid, pleural fluid, synovial fluid and Continuous Ambulatory peritoneal dialysis (CAPD) fluid are usually sterile but may be infected with various types of microorganisms, including bacteria in various clinical situations [1]. Presence of microorganisms in normally sterile body sites can lead to life-threatening infections. [2, 3]. For early and accurate diagnosis of these infections, such body fluids are cultured in appropriate enrichment media. [1, 2].

The relatively low counts of the bacterial pathogen in the fluid samples along with prior institution of empirical antibiotics often hinder the successful isolation of pathogens by conventional culture techniques. Another practical problem faced in the processing of these samples is that often the quantity of these samples is insufficient. All these factors contribute to lower rates of culture positivity by conventional culture procedures.

It is reported that inoculation of body fluids into the blood culture bottles simultaneous with conventional culture methods and processing them in the automated blood culture systems are useful in detecting the probable causative microorganisms. [1, 2].

Although the BacT/Alert system has been thoroughly evaluated for culturing of blood, only a limited number of studies have evaluated the utility of this method for culturing of other types of sterile body fluids [4, 5, 6]. The present study was designed to assess the performance of the BacT/Alert system to recover microorganisms from several types of sterile body fluids with standard aerobic bottles versus conventional media.

This study was designed to evaluate the ability of the BacT/Alert system to recover microorganisms from several types of sterile body fluids (other than blood and urine). The

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mean time to detection of growth by BacT/ALERT system was compared with that of the standard culture methods using solid plated media. The ability of the BacT/Alert to recover more number of organisms than the solid media was also assessed.

The BacT/Alert system is a continuously monitored blood culture system for detecting bacteremia and fungemia [7].

In this study, we aimed to compare the culture results of sterile body fluids processed simultaneously by both of conventional method and BACT/Alert blood culture system from 1st January 2016 to 31st December 2016 in microbiology laboratory retrospectively.

Brief background of the Project

This study is designed to evaluate the ability of the Automated blood culture system to recover microorganisms from several types of body fluids (other than blood and urine).

Although the Automated blood culture system has been thoroughly evaluated for culturing of blood, only a limited number of studies have evaluated the utility of this method for culturing of other types of sterile body fluids.

There is also a risk of growing contaminant microorganisms because of improper collection technique and improper transportation and sample storage.

Aims and Objective

The aim of this study is to:

Compare the culture results of body fluids processed simultaneously by both conventional method and automated blood culture system using BACT/Alert Blood Culture System.

MATERIAL AND METHODS

After ethical committee approval, a retrospective analytical study of one year duration (January 2016 to December 2016) was done. Because the study was done on the samples already sent to the laboratory and did not involve additional intervention to the patient, individual patient consent was waived.

Specimen: A total of 162 body fluid specimen obtained from patients of BARC Hospital, Anushakti Nagar, Mumbai were included in the study. The selected study period is from January 2016 to December 2016. All body Fluid specimens other than blood and urine received for culture and sensitivity testing in microbiology laboratory is included in this study. The specimens were processed for culture in the microbiology laboratory, both by conventional plate methods and by using the automated blood culture system. In our hospital we are using BacT/Alert blood culture bottles for Automated Blood culture system (BioMerieux, France)

Amongst the 162 specimen received, 10 Samples were rejected and not processed because of technical reasons or insufficient quantity and 4 samples were processed only by conventional method because of insufficient quantity. So 14 samples were excluded from the study and total 148 specimens was considered for study.

Conventional method: The samples were inoculated on 5 % blood agar and MacConkey agar and incubated at 37^0C for 48 hours and was observed for growth of bacterial colonies. Identification of the positive cultures was performed as per standard protocols of bacterial identification. [8] The cultures was declared sterile if there was no growth on the plates after 48 hours of incubation.

Automated (BacT/Alert) Blood Culture Method: 0.5 to 2 ml of specimen was inoculated into Bact/Alert standard aerobic bottles and was incubated in the BacT/Alert system for a maximum period of 96 hours. Standard BacT/Alert software was used for recording the results. Bottles flagged as positive by the BacT/Alert instrument were subcultured for aerobic organisms. The organisms was identified as per standard protocols.[8] Growth of Gram Positive Bacilli, Big GPC, Micrococcus Species and diphteroids were considered as growth of contaminant.

Statistical Methods

Qualitative Results of paired samples obtained from the study was proposed to be compared by Fisher's test and relative odd was obtained. The data obtained was also statistically analyzed using Microsoft Excel software package taking $p \leq 0.05$ as the cut off for significant result.

At the end of this study we:

- Analyze ability of the Automated blood culture system (BacT/Alert) to recover more number of organisms than the Conventional culture method.
- Find out false positive rate of Automated Blood culture system.
- Study mean time for growth of various types of microorganisms using BacT/Alert blood culture system.

RESULTS

In one year period a total of 148 fluid samples were processed the distribution of which has been shown in Table 1.

Table 1 Distribution of Body fluid

Type of fluid	Number of Sample Received
Pleural fluid	61
Ascitic fluid	38
Cerebrospinal fluids (CSF)	24
Continuous Ambulatory peritoneal dialysis (CAPD)	16
Synovial fluid	5
Peritoneal fluid	4
Total	**148**

Table 2 a Culture positivity rates for pathogens using the different methods

Type of fluid	Total no. of sample	Direct plating		BacT/Alert	
		Growth of Pathogens	%	Growth of Pathogens	%
Pleural fluids	61	1	1.64	16	26.23
Ascitic fluid	38	5	13.16	10	26.31
Cerebrospinal fluids (CSF)	24	0	0	2	8.33
Continuous Ambulatory peritoneal dialysis (CAPD)	16	1	6.25	5	31.25
Synovial fluid	5	0	0	0	0
Peritoneal fluids	4	0	0	4	100
Total	148	7	4.73	37	25.00

Table 2 demonstrates the comparative culture positivity rates using the direct plating and BacT/Alet Culture. The contaminants have been excluded while calculating the culture positivity rates.

Table 2 b Culture positivity rates for pathogens using the different methods

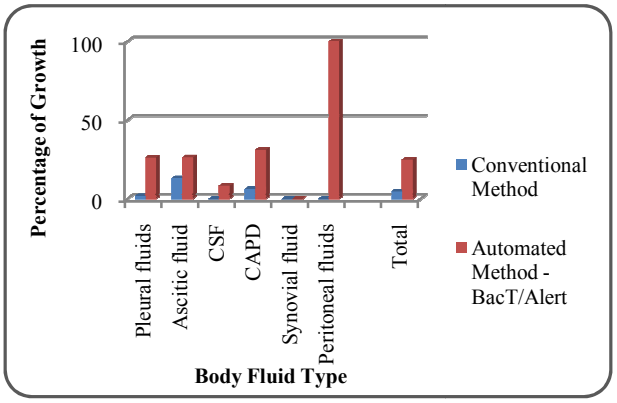


Table 3 demonstrates the comparative culture positivity rates using the direct plating and BacT/Alet Culture of the contaminants only.

Table 3 Culture positivity rates of contaminant using the different methods

Type of fluid	Total no. of sample	Direct plating		BacT/Alert	
		Growth of Contaminant	%	Growth of Contaminant	%
Pleural fluids	61	1	1.64	10	16.39
Ascitic fluid	38	2	5.26	4	10.53
Cerebrospinal fluids (CSF)	24	0	0	5	20.83
Continuous Ambulatory peritoneal dialysis (CAPD)	16	3	18.75	6	37.50
Synovial fluid	5	1	20.0	1	20.0
Peritoneal fluids	4	0	0	4	100.00
Total	148	7	4.73	26	17.57

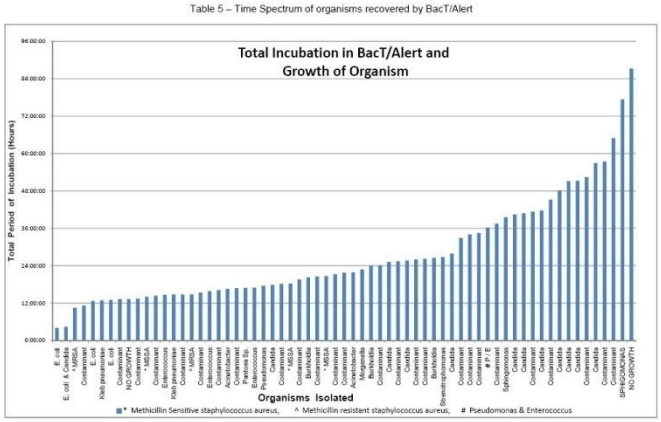
Table 4 demonstrates the Sample wise distribution of the isolates,

Table 4 Sample wise distribution of the isolates

Organisms isolated	Sample					Total Number of Isolates
	Ascitic fluid	Pleural fluid	CSF	CAPD Fluid	Peritoneal fluids	
Gram positive organisms						
• S. aureus	1	3	1	-	-	5
• Enterococcus spp	2	2	-	-	-	4
Enterobacteriaceae group						
• E. coli	3	-	-	1	-	4
• K. pneumoniae	-	1	-	-	1	2
Non-fermenter group						
•P. aeruginosa	1	1	-	-	-	2
•Acinetobacter spp	-	1	-	1	-	2
•Other non-fermenter.	1	5	-	-	2	8
Candida Spp.	2	4	1	4	1	12
Total number of isolates	10	17	2	6	4	39

There was mixed growth of two organisms in two samples. So total 39 organisms was isolated from 37 fluid samples.

Table 5 demonstrates the Time Spectrum of organisms recovered by BacT/Alert



DISCUSSION

Our study shows that culturing of normally sterile body fluids with both the conventional method and automated blood culture system simultaneously is an effective way to detect the probable present microorganism in the specimen. It is stated that this finding may be justified as follows :

1. more amount of the material is incubated into the blood culture bottle in comparison to the conventional method,
2. blood culture bottles which are enriched special media can support the growing of microorganism in low amounts,
3. the increasing chance of isolation of fastidious microorganisms with longer incubation period with blood culture systems and
4. the presence of naturalizing elements in blood culture bottles can prevent the inhibitory effect of antimicrobials probably used by the patient [2, 3].

In our study, automated blood culture system detected more culture positivity than the conventional method (P<0.001).

In our study 46.15 % of the samples grew Gram negative bacilli, the most common being those belonging to the non-fermenter group (12 isolates) followed by the Enterobactereaceae family (6 isolates).

Others who have studied the bacteriological profile of fluids have also reported a similar predominance of Gram negative organisms. Daur *et al*[3] reported that Pseudomonas aeruginosa and Escherichia coli were the most frequently isolated pathogens in their study. Similar finding were also reported by Lakshmi *et al*[9] who found that the enteric Gram negative bacilli were the predominant pathogens. Bobadilla *et al*[10] and Siersema *et al*[11] who evaluated culture methods in bacterial peritonitis, also reported a similar predominance of Gram negative flora. A recent study on spontaneous bacterial peritonitis also reported that majority of the isolates were Gram negative bacilli.[12]

Our study also demonstrated the usefulness of enrichment culture to improve the yield of pathogens from all types of body fluids. Table 2 shows that using BacT/ALERT, there was an overall increase in culture positivity rate to 25.00 % as compared to direct plating technique which had a culture positive yield of 4.73%. Other studies using automated culture

systems have also reported a significant increase in culture positivity rates after enrichment. Pal *et al*, in his study has reported a 36.96% isolation rate from body fluid using BACTEC system.[13] Other automated culture systems like BacT/Alert have also proved helpful in other studies. In the study by Lakshmi *et al*[9], they found an increased culture yield by 10.33% using BacT/ALERT culture over direct culture of body fluids like ascitic, pleural and pericardial fluid. Menzies *et al*[14] in 2011, has recently shown that for pleural fluids, enrichment in blood culture bottles was useful. In another recent study utilizing the BacT/Alert system, Yoon *et al*[15] has also reported a significant increase in culture positivity of peritoneal dialysis fluid (78.6%) as compared to the conventional method (50%).

Table 3 shows that using BacT/ALERT, there was an overall increase in growth of contaminants (17.57 %) as compared to direct plating technique (4.73%). Other studies using automated culture systems have also reported a significant increase in growth of contaminants after enrichment. However, presence of contaminants has to be correlated with patient's clinical findings including presence of immunosuppression as well as history of broadspectrum Antibiotic treatment.

Simor *et al*. [16] and von Essen *et. al*. [17] reported that blood culture systems significantly increased the isolation rate of contaminants.

Growth of contaminant microorganisms may be reduced by improving collection technique, proper transportation and proper sample storage and thus reducing the incidence of false positive reports.

Table 5 demonstrates the time Spectrum of organisms recovered by BacT/Alert. The mean time to detection of growth was <24 hours for bacterial growth and 24 to 48 hours for Candida Species.

CONCLUSION

In conclusion, our study indicates that the BacT/Alert system is a reliable and efficient system and a less labour-intensive approach to the early detection of clinically significant microorganisms in large volume aspirates and body fluids. In our study, Gram negative bacilli was predominantly recovered from most body fluids which was comparable with other studies reported in the literature. We also found that inoculation of body fluids into automated enrichment media improves the positive culture rate compared to the Conventional culture method. Automated systems significantly shorten the time to diagnosis, thus allowing early diagnosis and early administration of appropriate treatment; thereby curtailing the overall healthcare expenditure and morbidity of the patient. However, in resource-poor settings simple enrichment can be an effective alternative and help in improving the culture positivity of the fluid samples.

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